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Original Article

The Effect of Resistance Training on Levels of Interlukine-6 and High-Sensitivity C - reactive protein in Older-Aged Women

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A B S T R A C T

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Introduction: Aging is associated with elevated levels of some proinflammatory factors and exercise is a non-invasive intervention to improve immune function among older adults .The aim of the study was to compare resistance training effects on interlukine-6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) levels in older-aged women.

Methods: The study was quasi-experimental and forty healthy females were selected and randomly assigned to one of four groups: strength after endurance training (endurance + strength (E + S), n = 9), strength prior to endurance training (strength + endurance (S + E), n = 10), interval resistance-endurance training (Int, n = 12), and control (n = 9) groups. The training program was performed for eight weeks, three times per week. Human TNF-α and IL-6 sandwich ELISA Kit were used. Within-group differences were analyzed using a paired samples t-test and between-group differences were analyzed using one-way analysis of variance.

Results: The intra-session order had not significantly influence on the adaptive response of waist-to-hip ratio (p = 0.55), IL-6 (p = 0.55) and hs-CRP (p = 0.55) throughout the study. However, significant differences were shown following combined training between the S + E, E + S and Int groups for Vo2 max (p = 0.029), body mass (p = 0.016) and BMI (p = 0.023) when comparing pre and posttests.

Conclusion: This study confirmed that adaptations to a combination of endurance and resistance training appear to be independent of whether resistance training occurs prior to or following endurance training.

Keywords: Exercise, Inflammation, IL-6, hs-CRP, Aging

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Introduction

Aging is a process in which the physiological capacity of the body continuously declines after age 30. Given the shift in population, demographics shows increased numbers of elderly individuals above age 60 in Iran and structure of age pyramid is reversing (1).

Chronological aging itself is not a disease. Instead, aging is a process results from decline in physiological capacity of the body (2). Numerous theories, such as free radical, neuroendocrine, pineal gland, and immunological theories, have attempted to explain the decline in body function observed during aging process (3). It is generally believed that immune function declines with advancing age (4). As the body ages, age-associated immune deficiency begins and undergoes some deterioration known as immunosenescence (5) and causes some age-associated diseases such infectious diseases, coronary heart disease and stroke, diabetes type 2, Alzheimer's disease, and osteoarthritis (6). Chronic, low-grade inflammation is an independent predictor of several aging-related diseases (7). It is well known that aging is often associated with elevated levels of some proinflammatory factors such as tumor necrosis factor-α (TNF-α), interlukine-6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) (8-10).

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Among the various strategies to improve immune function in the elderly, exercise training is the best non-invasive interventions without negative side effects (11). Several review articles have been previously published and illustrated the potential effects of exercise on immunosenescence (12). It has been shown that weight loss is also a useful intervention for reducing TNF-α and IL-6 levels, because adipose tissue releases large amounts of the these factors (13, 14). In addition, the elevated CRP levels are associated with body fat (15).

However, very little is known regarding the interaction between concurrent training, aging and immune system, especially, concurrent training with different order sequences. For example, Rall et al. showed that a 12-weeks period of resistance training did not reduce TNF-α and IL-6 levels in elderly subjects (16). Several studies have shown that endurance exercise improves immune function and risk factors for chronic diseases in elderly individuals (17-20). However, nothing is known about the effect of concurrent training with different orders, Libardi et al. showed that 16 weeks of combined training in middle-age men has no effect on IL-6, TNF-α, and CRP levels (21). They also indicated that combined plus resistance training had no significant differences in TNF-α, IL6 and inflammatory biomarkers (22). Conraads et al. similarly illustrated that four months combined endurance/resistance training did not alter IL-6 and TNF-α levels (21). It seems that resistance training is associated with reduced risk of low grade inflammation related aging and long-term resistance training may be effective to protect against low-grade inflammation in the elderly (23). Recently, Stefanov et al. in a six-month combined exercise program reported a significant decrease in CRP in middle-aged women (24). In addition, Jorge et al. in an experimental study compared the effects of 3 different modalities of exercise training on inflammatory markers. They showed that there were no differences in CRP levels following aerobic, resistance, and combined exercise interventions (25).

For some time we have been interested in how much endurance and resistance training and what order can most be beneficial for acquiring health in elderly people. However, very little is known regarding the interaction between combined training, aging, and the immune system, particularly for combined training in different orders. Researchers were interested to determine the order of strength and endurance training that was most beneficial for regaining health-related physical fitness in elderly females. The best of our knowledge, there are no systemic published scientific studies that examined effects of order sequence of concurrent exercise on proinflammatory factors IL-6 and hs-CRP in aged women. The purpose of this study was to compare the effect of resistance training on pro-inflammatory markers levels IL-6 and hs-CRP in old-aged women.

Methods

Subjects

Forty eight old-aged healthy women (age; 55-70 years old) were recruited via telephony contact to a retirement center in Shahrekord city. The participants were required to be sedentary, defined as not having exercised for more than 20 minutes a week over the previous 6 months. Women taking any medication with chronic diseases or orthopedic limitation that could have interference effects on their participation in an exercise program or laboratory test results considered not eligible to participate in this study. Participants were familiarized with study procedures and were informed about the possible risks and benefits involved in this study both verbally and in writing. All subjects signed an informed consent for protection of human subjects. The subjects filled medical history questionnaire. They were assured that all questions contained will be kept strictly confidential. After baseline assessments, participants were assigned to control group (n = 9), resistance after aerobic training (E + S, n = 9), resistance prior to aerobic training (S + E, n = 10) and interval resistance-endurance (Int, n = 12) randomly. The exclusion criteria consisted of having a history of cardiovascular diseases, cancer, Hypertension, diabetes, thyroid disorders, addiction to tobacco, alcohol and drugs, endocrine disorders, kidney and liver diseases, surgery, and any intervention affecting the laboratory results. Therefore, eight participants withdrew from this study because of time constraints, lack of interest, personal and medical reasons unrelated to study such as a life-threatening event, hospitalization, significant disability, and surgical intervention.

Anthropometric measures

Body fat percentage was calculated from the value of 3-site skin fold test (abdominal, thigh and suprailiac), measured with a Lafayette Skinfold Caliper II. The body mass index (BMI) was calculated for each subject using the formula: BMI = weight (kilograms)/height2 (meters). Waist circumference (WC) was measured by using a flexible 2-meter standard tape measure at the maximal narrowing of the waist from anterior view. Hip circumference was measured at the point of maximal gluteal protuberance from the lateral view. Waist/hip ratio was calculated through dividing the waist circumference by hip circumference. Modified Bruce protocol treadmill test was used to measure aerobic power of subjects (26). The selected physical fitness test considered for this study was body composition (body mass index, BMI). The 1-RM leg press test (27) used to measure the lower limb strength capabilities and upper limb strength was measured using 1-RM bench press test (28).

Exercise training protocols

The Experimental groups underwent eight weeks combined (resistance plus endurance) training, three times a week. Each session consisted of 10 minutes

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for general warm up followed by 50 minutes of practicing, and then 10 minutes for cooling processes.

Blood Analysis

In order to examine serum IL-6 and hs-CRP levels, blood samples (10cc) from the subjects were collected 24 hours before exercise protocol and 48 hours after the last session of training program in 12-hour fasting state from the antecubital vein in a sitting position. Then blood samples were centrifuged for 10 minutes in 40C, 500×g to separate the serum. Human IL-6 sandwich ELISA Kit (Hangzhou Eastbiopharm Co, cat number CK-E10140), sensitivity= 0.13 pg/ml, and human hs-CRP sandwich ELISA Kit (Hangzhou Eastbiopharm Co, cat CK-E10968), sensitivity=0.025 ng/ml, were used to measure serum IL-6 and hs-CRP levels, respectively.

Ethical considerations

The Ethics Committee of Shahrekord University approved the study protocol with Code No: SH/93/108. Participants could withdraw from the study at any time, or in the case of experiencing a serious adverse event associated with exercise training voluntarily. An informed consent was signed and a medical history questionnaire was filled by all participants. They were assured about confidentiality of their information. The protocol of this trial was registered in the Iranian website (http://www.irct.ir) for registration of clinical trials (IRCT code: IRCT2014123019995N3).

Statistical analysis

All values are represented as mean ± SD. For testing the normality of distribution, Kolmogorov-Smirnov test was used. Data were analyzed by dependent T test to compare pre-test and post-test in each group. A one-way ANOVA test was used to compare the amount of changes in experimental and control training groups after eight weeks.

Results

The results were based on the observations of 16 people in control, 9 people in E + S, 10 people in S + E and 12 people in Int groups who completed the study. The results after eight-week combined training are presented in Tables 1.

The data showed that all experimental groups had a significant decrease in body mass and body fat percentage [E + S ($p < 0.017$), S + E ($p < 0.003$) and Int ($p < 0.000$)], while the control group did not change ($p < 0.51$). There was significant between-group difference between two training groups ($p < 0.017$). one-way variance and Toki- following test showed significant difference in body mass between four groups , specially between Con and S+E groups ($p < 0.02$), and Con and Int groups ($p < 0.03$).

Decreases in BMI occurred in all groups [E + S ($p < 0.005$), S + E ($p < 0.003$) and Int ($p < 0.001$)]. Body fat percentage decreased in all training groups [E + S ($p < 0.001$), S + E ($p < 0.001$) and Int ($p < 0.001$)]. There were significant differences between the E + S and S + E, Int and Con groups ($p < 0.023$).

WC decreased from pre- to post-measurements E + S ($p < 0.001$), S + E ($p < 0.008$) and Int ($p < 0.003$)]. While Changes in the control group were not significant ($p < 0.22$).

Body fat percent decreased in all intervention groups. Body fat percentage decreased in the E + S ($p < 0.001$) and S + E ($p < 0.001$) and Int group ($p < 0.001$). There were no between-group differences ($p < 0.08$).

Minor changes were observed in WHR. In within-group analysis, E + S ($p < 0.17$) S + E ($p < 0.80$) and Int ($p < 0.32$) did not change WHR during the period. Training protocols increased VO2max significantly in all training groups [E + S ($p < 0.003$), S + E ($p < 0.003$) and Int ($p < 0.024$)]. Results of one-way ANOVA and LSD post-hog tests showed that VO2max was significantly different after training programs ($p < 0.029$). VO2max was also significantly higher in S + E than Con groups ($p < 0.01$).

The effects of the program on inflammatory markers are shown in Table 1. IL-6 serum concentrations were not significantly changed in [E + S ($p < 0.75$), and Int ($p < 0.097$)] except for the S + E training group ($p < 0.043$). Differences were not found for the hs-CRP levels in the all groups E + S ($p < 0.057$), S + E ($p < 0.131$), Int ($p < 0.112$) and Con ($p < 0.694$).

Discussion

There have been many studies that examine the influence of endurance and resistance exercise on some inflammatory factors. But, to our knowledge, this is the first study that examines the influence of manipulating the order sequence of the concurrent training on adaptations of inflammatory mediators. Few studies have investigated effect of concurrent resistance and endurance training on elderly populations (29-32).

All experimental groups showed decreases in body mass, BMI, body fat percent, WC and WHR. It seems that through involvement of strength and endurance, this kind of training provided a greater stimulus and reduced body fat percentage and body mass in aged women. The literature provides evidence that there was a relationship between energy expenditure in physical exercise training and the lean body mass (33). They illustrated that concurrent training was more efficient to decrease body fat percentage when compared with resistance and endurance training alone (34-36). Antunes et al. found that combined aerobic and resistance exercise training is effective for burning of body fat percentage in obese adolescents (33). Furthermore, Ghahramanloo et al. showed that eight weeks of combined training improved body fat percentage in young men (37).

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Table 1. The comparison of changes in the measured variables before and after eight weeks of exercise interventions

Variables	Groups*	Mean ± SD		p-value within	p-value Between
		Pre- test	Post-test	Group	Groups
Body mass (kg)	E + S	74.66 ± 4.68	72.77 ± 4.67	0.005	0.017
	S + E	70.80 ± 3.90	68.60 ± 3.86	0.003	
	Int	66.41 ± 2.69	64.41 ± 2.44	0.000	
	Con	76.88 ± 3.78	76.66 ± 4.05	0.51	
BMI (kg/m2)	E + S	29.89 ± 1.20	29.12 ± 1.21	0.005	0.023*
	S + E	29.23 ± 1.71	28.30 ± 1.56	0.003	
	Int	27.57 ± 0.92	26.76 ± 0.86	0.000	
	Con	31.75 ± 0.91	31.63 ± 1.01	0.42	
% Body Fat	E + S	30.49 ± 1.0	26.90 ± 1.47	0.000	0.08
	S + E	31.66 ± 1.35	27.77 ± 1.30	0.000	
	Int	30.65 ± 1.05	27.88 ± 0.95	0.000	
	Con	28.50 ± 0.92	27.50 ± 1.0	0.08	
WC (cm)	E + S	98.33 ± 3.08	93.44 ± 3.03	0.000	0.006**
	S + E	95.40 ± 3.08	92.50 ± 3.18	0.008	
	Int	93.50 ± 2.64	90.25 ± 3.08	0.003	
	Con	97.44 ± 4.36	97.00 ± 4.53	0.22	
(WHR)	E + S	0.91 ± 0.01	0.89 ± 0.01	0.17	0.55
	S + E	0.88 ± 0.01	0.88 ± 0.01	0.80	
	Int	0.92 ± 0.01	0.91 ± 0.02	0.32	
	Con	0.88 ± 0.02	0.88 ± 0.02	0.83	
Vo_2max (ml/kg/min)	E + S	29.07 ± 1.88	34.01 ± 2.05	0.003	0.029*
	S + E	24.60 ± 1.35	31.81 ± 1.05	0.003	
	Int	23.70 ± 1.78	27.93 ± 2.18	0.024	
	Con	24.77 ± 3.03	24.25 ± 3.01	0.43	
IL-6 (pg/ml)	E + S	16.77 ± 8.2	12.65 ± 7.5	0.75	0.43
	S + E	45.48 ± 15.41	10.00 ± 6.28	0.043	
	Int	30.91 ± 12.36	6.22 ± 2.42	0.097	
	Con	36.25 ± 14.03	22.53 ± 14.88	0.327	
hs-CRP (mg/L)	E + S	9.94 ± 3.84	6.95 ± 3.20	0.057	0.553
	S + E	9.61 ± 3.41	6.12 ± 2.40	0.131	
	Int	6.21 ± 1.83	3.39 ± 8.26	0.112	
	Con	4.3 ± 1.25	4.69 ± 7.25	0.694	

*E+S: Resistance after aerobic training; S+E: Resistance prior to aerobic training; Int: Interval resistance-endurance; Con group: subjects who not participated in exercise training; BMI: body mass index. WC: Waist circumference. WHR: circumference waist of hip. *: Significant difference between two groups ($p < 0.05$). ** Significant difference between two groups ($p < 0.01$).

The data showed no significant between-experimental group difference (order effect) in body mass, BMI, WC and WHR following eight weeks of combined training. In accordance with our research, Vilacxa et al. illustrated that there was no difference during performing concurrent training in different sequences for oxygen consumption (37). In addition, Cardo et al. showed that sequence order of combined strength and endurance training had no influence on body fat percentage of elderly men (38).

The results of this study support findings of Küüsmaa that independent of the order of training, 24 weeks of concurrent training led to significant decrease in total body mass and body fat percent (39). Furthermore, irrespective of the order sequence, no differences were found in the body composition improvements between groups. It seems that longer training courses may show greater differences between groups with different orders in our study.

In our study, concurrent training, independent of order, has been shown to increase aerobic power compared with control group. In accordance with our research, some researchers illustrated that combining endurance and strength training into the same training session improved aerobic power more effective than either alone (36, 40-42).Few studies have shown the influence of order sequence of endurance and resistance training on VO2max.Our study is inconsistent with some researches reporting exercise sequence might be an important variable in the

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adaptations to the concurrent training program. Chtara et al. illustrated that aerobic power improvements are greater in young men when strength training was performed before endurance (43). In another study, Chtara et al. showed that Circuit resistance training immediately after endurance training (E+S) produced greater improvement in the endurance power and aerobic capacity than the versus order (S+E) (41). It should, however, be noted, that there were no significant differences between the groups in training induced adaptations aerobic power in present study.

It seems that combining resistance and endurance training does not interfere with the development of aerobic power in the aged women. Similar results have been found in more recent studies. Schumann et al. illustrated that there was no inter-group difference between groups with different sequence training in aerobic capacity (42). Furthermore, the current data is consistent with that in the study by Cadore et al (38). These authors found no differences in VO2max between SE and ES in elderly men following 12 weeks of concurrent training.

The results of our study indicated that serum hs-CRP and IL-6 concentration unchanged after eight weeks of concurrent training with different order in aged women, except the IL-6 in S+E training group.

It seems that the beneficial effect of combined training was not associated with hs-CRP and IL-6 reduction, which is in line with other studies (22, 46). Probably longer period that reduces visceral fat is required for lowering systemic inflammation [46]. Indeed, Balducci et al. illustrated a reduction in hs-CRP and IL-6 and an increase after 12 months of exercise training, whereas no significant changes were found in the first three months of training(44). Indeed, other factors which significantly change with exercise training are more powerful determinants of CRP concentrations than of IL-6 and TNF- α.

This finding is inconsistent with Jorge et al. who concluded that concurrent training significantly decreased CRP concentration in patients with type 2 diabetes mellitus (25). Furthermore, Touvra et al. illustrated that concurrent resistance and endurance training improved CRP concentration in patients with type 2 diabetes without altering the levels of IL-6 and TNF-α (45). Libardi et al. verified that 16 weeks of concurrent training could increase functional capacity, but did not improve inflammatory biomarkers (IL-6 and TNF-α), except serum hs-CRP concentration in middle-aged men.

According to these findings, Beavers et al. found that one year of combined aerobic, strength, balance, and flexibility training, did not improve body mass, CRP and TNF- α in elderly men and women. As we showed in our study, there are no significant reductions in body fat percentage and chronic inflammatory factors. Possibly, exercise interventions effectively improve chronic inflammation only in association with concomitant body fat loss. Because, there is a strong correlation between hs-CRP and body fat percentage (46).

Furthermore, the mechanisms underlying concurrent training-induced reduction of serum hs-CRP occurring with combined training may include a decrease in some cytokines production by reducing adipose tissue (47). In the present study, no differences in inflammatory biomarkers were found between all concurrent intervention groups.

Conclusion

In summary, despite the fact that some studies showed that aerobic and resistance training interventions could reduce insulin resistance in middle-aged women, but our study could not prove that this kind of intervention could be an effective treatment to improve chronic inflammatory marker levels. Our results highlighted that combined aerobic training and resistance training, independent of order sequence is an effective training method to reduce body fat and BMI in old-aged women.

Study limitations

Lack of long term follow up post interventions is a certain limitation of this study. Another limitation of this study is that improvements in some body composition variables such as WHR, WC and body mass might not only be due to exercise training, because it is possible that patients alter their diet behavior in this study. It remains unclear whether or not patients altered their diet. Because we did not quantified the energy intake of them in the present study and there was no information about appetite changes following interventions.

Conflict of interest

The authors declare that they have no competing interests.

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